SANTA CRUZ BIOTECHNOLOGY, INC.

Cytoplasmic CysRS siRNA (h): sc-77084

BACKGROUND

Aminoacyl-tRNA synthetases consist of a family of enzymes that catalyze the specific aminoacylation of tRNA by their cognate amino acid in the initial step of ribosome-dependent protein biosynthesis. Cytoplasmic CysRS (Cysteinyl-tRNA synthetase, cytoplasmic), also known as CARS, is a 748 amino acid member of the class-I aminoacyl-tRNA synthetase protein family. Cytoplasmic CysRS is a monomeric protein that binds one zinc ion per subunit for use as a cofactor. Cytoplasmic CysRS uses ATP to convert L-cysteine and tRNA(Cys) into ADP, a diphosphate and L-cysteinyl-tRNA(Cys). A chromosomal aberration of the gene that encodes Cytoplasmic CysRS is associated with inflammatory myofibroblastic tumors (IMTs). Cytoplasmic CysRS is expressed as two isoforms produced by alternative splicing events.

REFERENCES

1. Cruzen, M.E., et al. 1993. Assignment of the cysteinyl-tRNA synthetase gene (CARS) to 11p15.5. Genomics 15: 692-693.
2. Kim, J.E., et al. 2000. An elongation factor-associating domain is inserted into human cysteinyl-tRNA synthetase by alternative splicing. Nucleic Acids Res. 28: 2866-2872.
3. Davidson, E., et al. 2001. Isolation of two cDNAs encoding functional human cytoplasmic cysteinyl-tRNA synthetase. Biol. Chem. 382: 399-406.
4. Cools, J., et al. 2002. Identification of novel fusion partners of ALK, the anaplastic lymphoma kinase, in anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor. Genes Chromosomes Cancer 34: 354-362.
5. Rush, J., et al. 2005. Immunoaffinity profiling of tyrosine phosphorylation in cancer cells. Nat. Biotechnol. 23: 94-101.
6. Evilia, C. and Hou, Y.M. 2006. Acquisition of an insertion peptide for efficient aminoacylation by a halophile tRNA synthetase. Biochemistry 45: 6835-6845.
7. Hohn, M.J., et al. 2006. Emergence of the universal genetic code imprinted in an RNA record. Proc. Natl. Acad. Sci. USA 103: 18095-18100.
8. Liu, C., et al. 2007. Kinetic quality control of anticodon recognition by a eukaryotic aminoacyl-tRNA synthetase. J. Mol. Biol. 367: 1063-1078.

CHROMOSOMAL LOCATION

Genetic locus: CARS (human) mapping to 11p15.4.

PRODUCT

Cytoplasmic CysRS siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Cytoplasmic CysRS shRNA Plasmid (h): sc-77084-SH and Cytoplasmic CysRS shRNA (h) Lentiviral Particles: sc-77084-V as alternate gene silencing products.

For independent verification of Cytoplasmic CysRS (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-77084A, sc-77084B and sc-77084C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Cytoplasmic CysRS siRNA (h) is recommended for the inhibition of Cytoplasmic CysRS expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

Cytoplasmic CysRS (A-3): sc-390230 is recommended as a control antibody for monitoring of Cytoplasmic CysRS gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Cytoplasmic CysRS gene expression knockdown using RT-PCR Primer: Cytoplasmic CysRS (h)-PR: sc-77084-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.